USER MANUAL

Reaction Modelling for Kinetic Processes

Jplus consulting
multivariate analytical technologies

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SYSTEM REQUIREMENTS AND INSTALLATION

Please refer to the 'System Requirements and Installation Guide' available on our website, and included with the ReactLab™ download.

CONTACT INFORMATION AND SUPPORT

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REFERENCE GUIDE

INTRODUCTION

ReactLab™ KINSIM provides extensive reaction modelling capabilities and allows visualisation and quantitative simulation of complex reaction mechanisms.

The program comes in both a free version, ReactLab™ KINSIM, and a licensed version, ReactLab™ KINSIM PLUS. The PLUS version offers extended graphics and visualisation features from the ReactLab GUI and a Parameter Slider feature which allows dynamic adjustments to reaction parameters to be observed interactively. The free version of ReactLab™ KINSIM also limits the available number of reaction steps which can be modelled to six.

	ReactLab KINSIM	ReactLab KINSIM PLUS
Max Reaction Steps	6	20
Interactive Parameter sliders	No	Yes
Extended graphics features and print support	No	Yes

In this manual which is applicable to both programs sections referring to features available only in ReactLab™ KINSIM PLUS will be preceded with *PLUS START* and appended *PLUS FINISH*

The programs, including all algorithms and the GUI front end has been developed in Matlab® and compiled to produce the final deployable application. They require the Matlab Compiler Runtime (MCR) library to be installed on the same computer. This allows the programs to run on computers without a standard Matlab installation and is supplied as part of the installation package.

Model entry and computational output are organised in Excel Workbooks, which are launched from, and dynamically linked to, the ReactLab™ application. Similarly, all numerical analysis options are organised in the Excel format. It is therefore necessary for Excel to be installed on the same computer as ReactLab™.

The benefit of using Excel is that all simulation results can be easily shared and further manipulated using standard Excel tools without further need for ReactLab KINSIM.

Excel workbooks are compatible with both the free and PLUS versions of ReactLab KINSIM.

The program requires Excel analysis workbooks to retain a strict format, as is provided in the examples and templates.

EXAMPLES

A selection of example workbooks and a template KINSIM workbook accompanies the program in a subfolder in the installation directory. We recommend you copy this subfolder to your documents library. Feel free to experiment with the parameters in the examples.

These include three simulations of an enzyme mechanism (Enzyme_1.xlsx, Enzyme_2.xlsx and Enzyme_3.xlsx); a reversible second order reaction (AplusBeqC.xlsx); a simulation of the Lotka-Volterra predator prey model (Lotka_Volterra_predator_prey_model.xlsx); the Belousov-Zhabotinsky cyclic reaction (Belousov-Zhabotinsky.xlsx) and a metal ligand complexation reaction which includes both kinetically observable rates and rapid protonation equilibria (Cu_cyclam.xlsx).

EXCEL TEMPLATES

To inspect an Excel ReactLab™ KINSIM workbook load it directly into Excel, or via the 'Load Excel' button in the ReactLab™ GUI.

The workbook is pre-formatted containing two worksheets, the '**Main'** sheet provides the purpose designed model and parameter entry interfaces. The '**About**' sheet provides contact information and workbook format version.

STARTING THE MATLAB PROGRAM

When ReactLab™ is launched it also requires the Matlab MCR to initialise. This can take a while and is very much dependant on computer performance, after which the ReactLab™ GUI appears as in Figure 1.

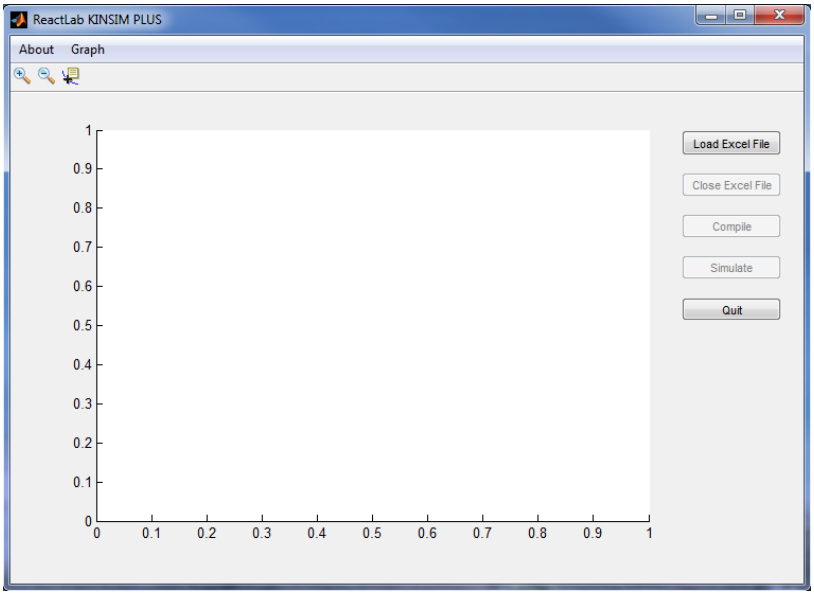


Figure 1: ReactLab KINSIM PLUS GUI control panel at startup

This GUI provides the main control interface for the program with a series of pushbuttons on the right hand side for key functions. These provide all the ReactLab™ program commands. Their operation is described over the following pages.

Note depending on the workbook status, some of these functions may be disabled.

The extra graphics presentation functionality in ReactLab KINSIM PLUS is accessed via the Graph menu item above the toolstrip.

An **About** menu item at the top left provide details of the ReactLab™ version and in the case of ReactLab™ KINSIM PLUS important licensing information.

PROGRAM OPERATION

Load Excel File

Press **Load Excel File** in the ReactLab™ GUI to select and link to an Excel Workbook. This will launch a new instance of Excel and load the requested workbook independent of any other open Excel workbooks. Only the ReactLab™ KINSIM workbook template or already developed spreadsheets can be loaded. Figure 2 shows the opened enzyme_1.xlsx example workbook 'Main' worksheet.

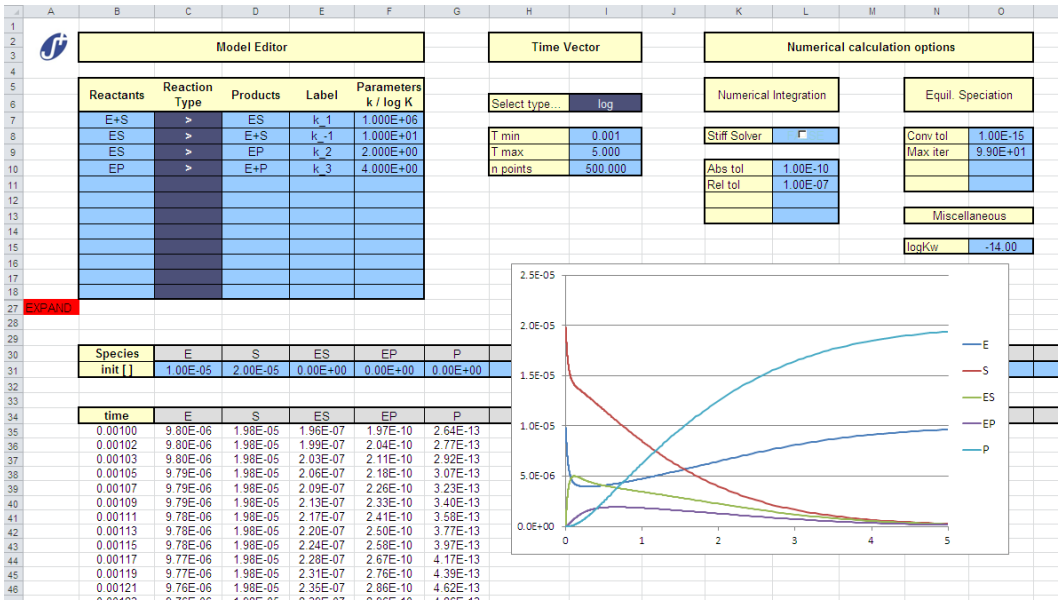


Figure 2: The Enzyme_1.xlsx workbook

Important: Excel is launched as an ActiveX server in an independent process linked to ReactLab™ which communicates with it through its Microsoft component object model (COM) interface. The launch of Excel and ActiveX server linkage is initiated by the Matlab (ReactLab) program. Linking to an already open workbook in Excel is **NOT** supported. If a workbook is prepared for simulation it should first be saved and re-opened from the running ReactLab™ application. Note all Excel functionality is available to manipulate the workbook as normal while it is linked to ReactLab™.

Close Excel File

This will close the current workbook and link, prior to opening another. If any changes have been made to the workbook the user must first switch focus to the workbook where he/she will be prompted as to whether the changes to the workbook need to be saved.

MODEL ENTRY AND COMPILATION

To demonstrate the simulation of new data we start with the pre-formatted template, ReactLab_KINSIM_Template (see Figure 3).

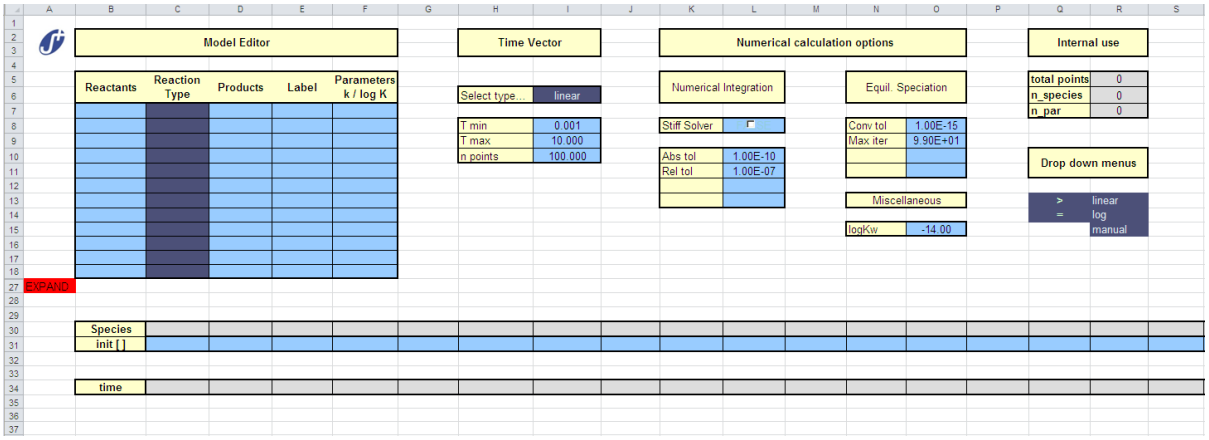


Figure 3: The ReactLab™ KINSIM template, *ReactLab_KINSIM_Template.xlsx*

The reaction we will model is the simple consecutive reaction scheme

$$\mathbf{A} \xrightarrow{k_1} \mathbf{B} \xrightarrow{k_2} \mathbf{C}$$

The reaction scheme is entered in the model entry area of the **'Main'** worksheet, see Figure 4. The species names and reaction types (using drop down list options) are added to the list in the fields headed 'Reactants', 'Reaction Type' and 'Products'. Note each reaction step is entered individually **A→B** and **B→C** which allows for a one-to-one correspondence of each reaction step to its parameter value in the 'Parameter' column. Parameters values and labels are not required for compilation.

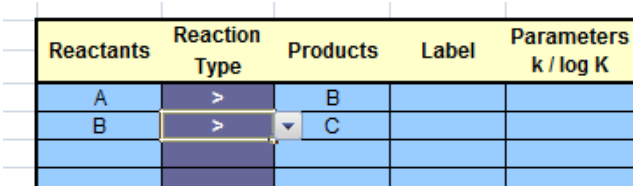
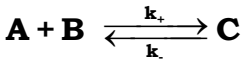


Figure 4: Entering the A→B→C model

A second order reaction **A+B→C** would be entered as:

Figure 5: Syntax for a 2nd order step

If the reaction is reversible with individual forward and reveres rates this is entered as two lines using the following syntax. Note the reverse reaction is actually entered 'backwards'.



A+B	>	C
C	>	A+B

Figure 6: Syntax for a reversible reaction

If a reaction comprises a rapid (effectively instantaneous) equilibrium it is entered using the '=' symbol. This is typically the case for protonation equilibria.

L+H	=	LH

Figure 7: Using the '=' symbol for a rapid equilibrium

In this case the parameter is an equilibrium constant; an equilibrium constant is always entered as its logarithmic (base 10) value. Any fast equilibrium is always entered syntactically as an association constant K_{ass} with the associated product on the right.

In addition leading numbers prefixing a species letter or string will be interpreted as stoichiometry coefficient for the species in question e.g. 2A > B. Similarly trailing numbers are used to represent multiple species in a particular complex e.g. ML, ML2, ML3 etc. Essentially, 'normal' chemical reaction equations can be used. For further information on the syntax rules see page 18.

In all cases a label for each parameter can be provided. This allows easier reference to mechanisms with multiple steps. These labels and the parameter values themselves are not required prior to compilation (see C below). Figure 11 gives an example of annotated rate and equilibrium constants.

This relatively simple syntax allows accurate models of realistic complexity to be correctly modelled (see Examples).

Important: It is necessary for any new entry in the Excel workbook to be properly completed, i.e. by hitting return or pressing the arrow or return key to take the focus away from the cell in question once the value has been assigned, otherwise the 'Incomplete worksheet entry' warning will be raised. Failing to enter values properly prevents ReactLab™ from accessing the worksheet cell through the ActiveX interface.

Compile Model

When model entry is complete press the **Compile Model** button.

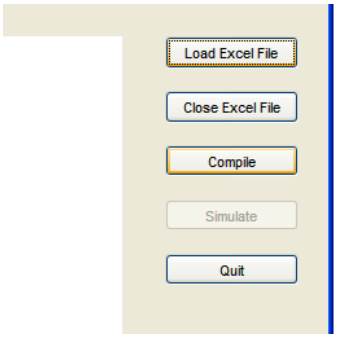


Figure 8: The compile button translates the model into the internal coefficient notation required for Kinsim to complete further computations

ReactLab™ reads in the model and translates it into an internal coefficient format necessary for its subsequent numerical calculations.

Initial Concentrations

The compilation process also extracts all the intermediate species names and lists them in a row in the **'Main'** Excel worksheet. This allows the convenient entry of the initial concentrations to be inserted, see Figure 9. Note that these concentrations are always defined for time zero, not the first time point at which the concentrations are calculated.

Species	A	B	C		
init [ ]	1.00E-01	0.00E+00	0.00E+00		

Figure 9: The initial concentration for all species are entered in the appropriate cells

Certain key values are calculated automatically by Excel and are required by ReactLab™, namely the number of reactions specified in the model for which there is a rate or equilibrium constant field (**n_par**) and the number of individual chemical species in the mechanism (**n_species**). Do not overwrite the formulae in these cells (they are protected in the templates supplied).

	Q	R
	Internal use	
	total points	100
	n_species	3
	n_par	2

Figure 10: Model information automatically generated by Excel

Constant entry

Prior to simulation estimates or known values for all rate (and equilibrium) constants need to be entered in the appropriate fields (Figure 11). Rates constants are entered as absolute

values but for equilibrium constants the parameter value must be expressed as its log value; e.g. 3 for $K_{ass}=10^{3}M^{-1}$.

Reactants	Reaction Type	Products	Label	Parameters k / log K
A	>	B	k_1	2.000E-01
B	>	C	k_2	1.000E-02

Figure 11: Annotated parameter value entry alongside the model in the 'Main' sheet

Timebase

Before the simulation can be completed, information about the time span for which the reaction should be simulated has to be provided. ReactLab KINSIM allows three different modes to define the time vector at which the concentrations will be computed:

- Linear
- Logarithmic
- Manual

The preferred time-base mode is selected from the drop-down menu.

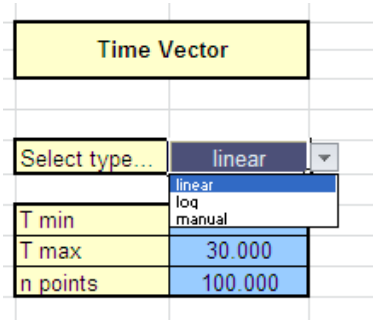


Figure 12: The time base is chosen from a drop-down menu

In the *linear mode* n_points are chosen equidistantly between the T_min and T_max parameters.

In the *logarithmic mode* each consecutive point is a constant factor times the previous point creating a logarithmin progression of time values. This factor is computed in such a way that there are n_points between the T_min and T_max parameters. In logarithmic mode the initial time point cannot be zero (if a zero value is provided the program will, by default, replace this with 1e-4).

In the *manual mode* the time points can be defined individually in the appropriate column. In this mode the entries for T_min, T_max and n_points are ignored

time	A
1.000	
2.000	
3.000	
5.000	
6.000	
8.000	

Figure 13: Manual entry for a specific time vector

Important note: irrespective of the time base and the initial time of the time vector, the initial concentrations defined as in Figure 9, are always defined for time zero.

Please note generation of the new time base requires the simulation step (below).

Simulate

After definition of the model, the initial concentrations and the time vector, the Simulation button starts the computation of the concentration profiles which are written into the appropriate columns of the spreadsheet.

Reactants	Reaction Type	Products	Label	Parameters k / log K
A	>	B	k_1	2.000E-01
B	>	C	k_2	1.000E-01

Select type...	linear
T min	0.000
T max	50.000
n points	51.000

XPAND

Species	A	B	C
init [ ]	1.00E-01	0.00E+00	0.00E+00

time	A	B	C
0.000	1.00E-01	2.00E-06	1.00E-11
1.000	8.19E-02	1.72E-02	9.06E-04
2.000	6.70E-02	2.97E-02	3.29E-03
3.000	5.49E-02	3.84E-02	6.72E-03
4.000	4.49E-02	4.42E-02	1.09E-02
5.000	3.68E-02	4.77E-02	1.55E-02
6.000	3.01E-02	4.95E-02	2.04E-02
7.000	2.47E-02	5.00E-02	2.53E-02

Figure 14: The computed concentrations are stored in the appropriate columns

The concentration profiles are also displayed in the KINSIM GUI as shown below.

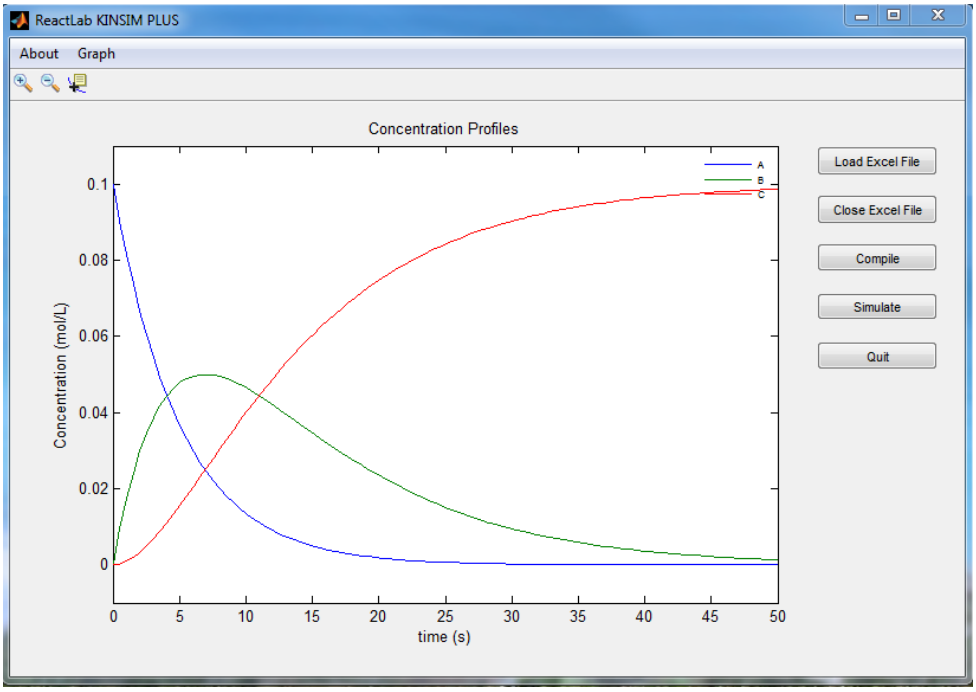


Figure 15 The KINSIM GUI once the simulation is completed

PLOTS IN EXCEL

There are several advantages of using excel as the user interface; a significant one is the power of Excel to produce your own preferred graphical output. For all plotting and graphing options we refer the reader to the Excel help and many textbooks. For our purposes the plot of the computed concentration profiles is relatively simple. A standard scatter plot is produced by selecting the relevant section of the spreadsheet:

time	A	B	C
0.000	1.00E-01	2.00E-06	1.00E-11
1.000	8.19E-02	1.72E-02	9.06E-04
2.000	6.70E-02	2.97E-02	3.29E-03
3.000	5.49E-02	3.84E-02	6.72E-03
4.000	4.49E-02	4.42E-02	1.09E-02
5.000	3.68E-02	4.77E-02	1.55E-02
6.000	3.01E-02	4.95E-02	2.04E-02
7.000	2.47E-02	5.00E-02	2.53E-02
8.000	2.02E-02	4.95E-02	3.03E-02
9.000	1.65E-02	4.83E-02	3.52E-02
10.000	1.35E-02	4.65E-02	4.00E-02
11.000	1.11E-02	4.44E-02	4.45E-02
12.000	9.07E-03	4.21E-02	4.88E-02
13.000	7.43E-03	3.97E-02	5.29E-02
14.000	6.08E-03	3.72E-02	5.68E-02

Figure 16: Selecting the relevant section of the spreadsheet

Followed by selecting the appropriate scatter plot:

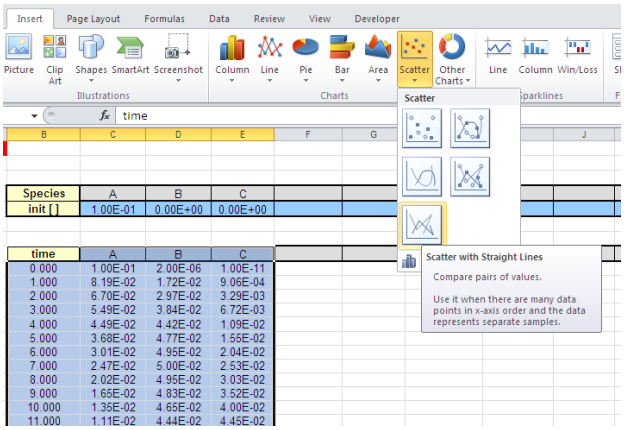


Figure 17: Selecting the appropriate Scatter plot

Results in the standard excel plot:

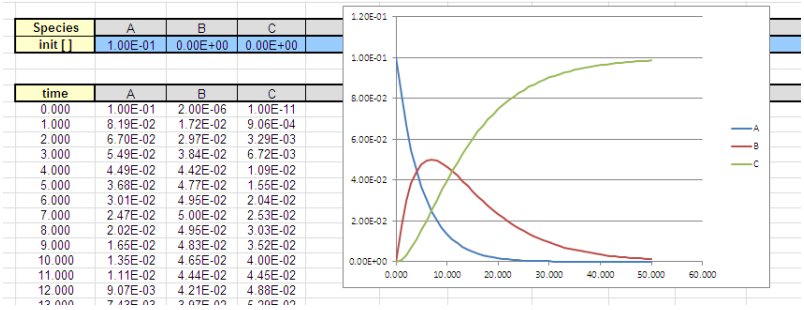


Figure 18: The resulting basic excel Scatter Plot reproducing the KINSIM GUI plot of Figure 15

Excel allows any number of annotations and formatting changes:

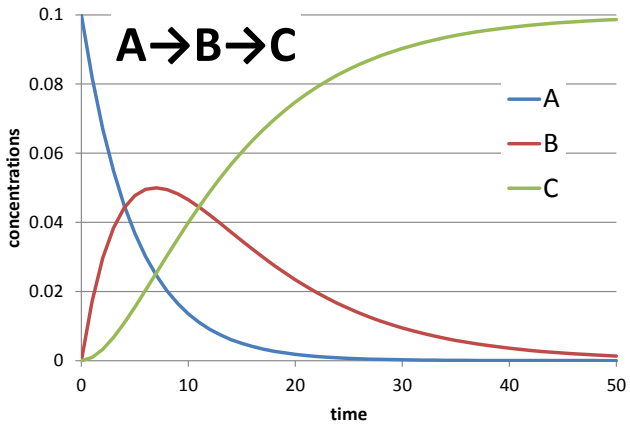


Figure 19: An example of an annotated Excel graph

PLUS START

GRAPH MENU

This menu is disabled in the free version of KINSIM and is available above the toolbar on the KINSIM PLUS GUI.

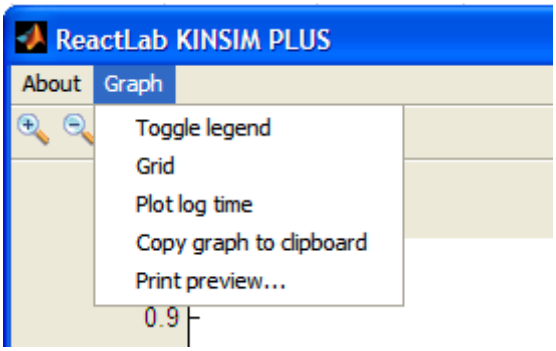


Figure 20: The Graph Menu in KINSIM PLUS

Toggle legend

Plots the figure legend outside the graphics area to improve trace visibility.

Grid

Plots a grid on the graphics background.

Plot log time

Switches between logarithmic and linear display of the time base.

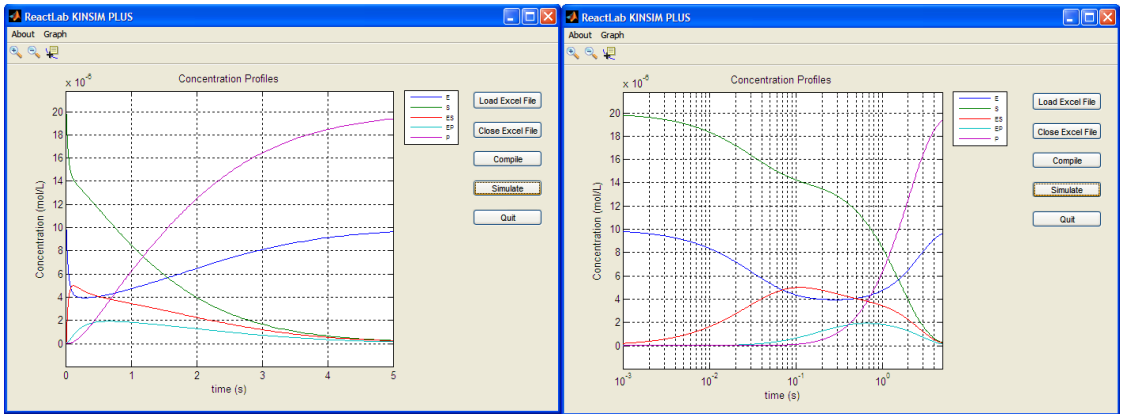


Figure 21: KINSIM graphs with grid and logarithmic time base

Copy graph to clipboard

Copy the graph to the clipboard and a Windows meta-file which can then be directly pasted to other documents.

Print preview

Print preview display allowing the graph to be resized and positioned prior to printing.

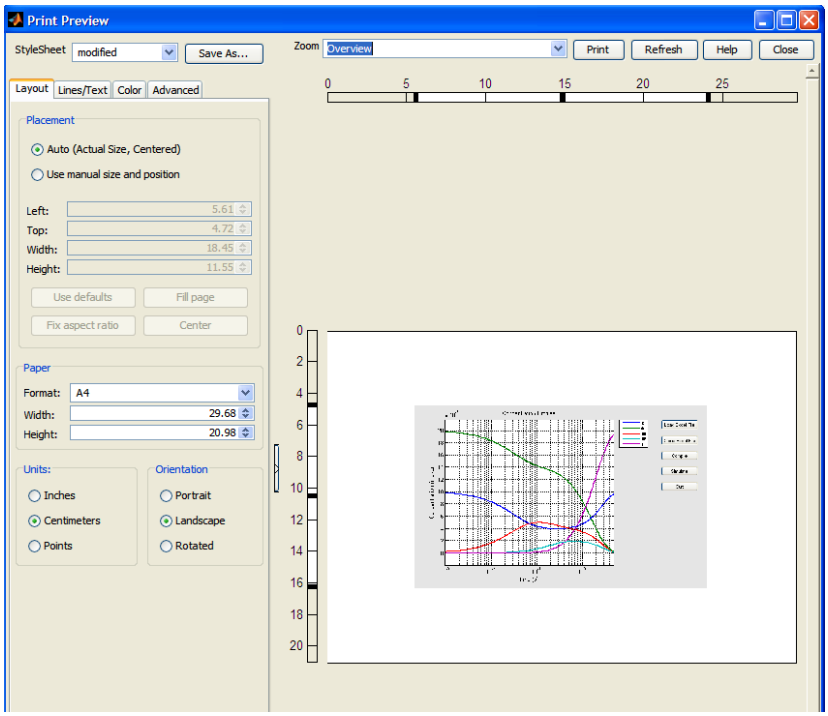


Figure 22: Print preview

PARAMETER SLIDERS

The parameter slider dialog box (see Figure 23) is only available as a feature of ReactLab™ KINSIM PLUS and allows dynamic simulation of the concentration profiles while interactively adjusting the parameters using the sliders.

Each rate or equilibrium parameter has a slider which allows the adjustment of the value. As soon as the change is made the corresponding values in the Excel workbook change accordingly and all the graphs are updated.

If a new value is entered in the edit box the sliders are re-centred around this new value. In this way the range span can be changed as required.

Note the rate value slider position is not centred since the span range is set to be from one fifth of the current value to five times the value and the slider range is linear (from the minimum to maximum). Conversely, the sliders are central for the logK parameters as these are adjustable by plus or minus five.

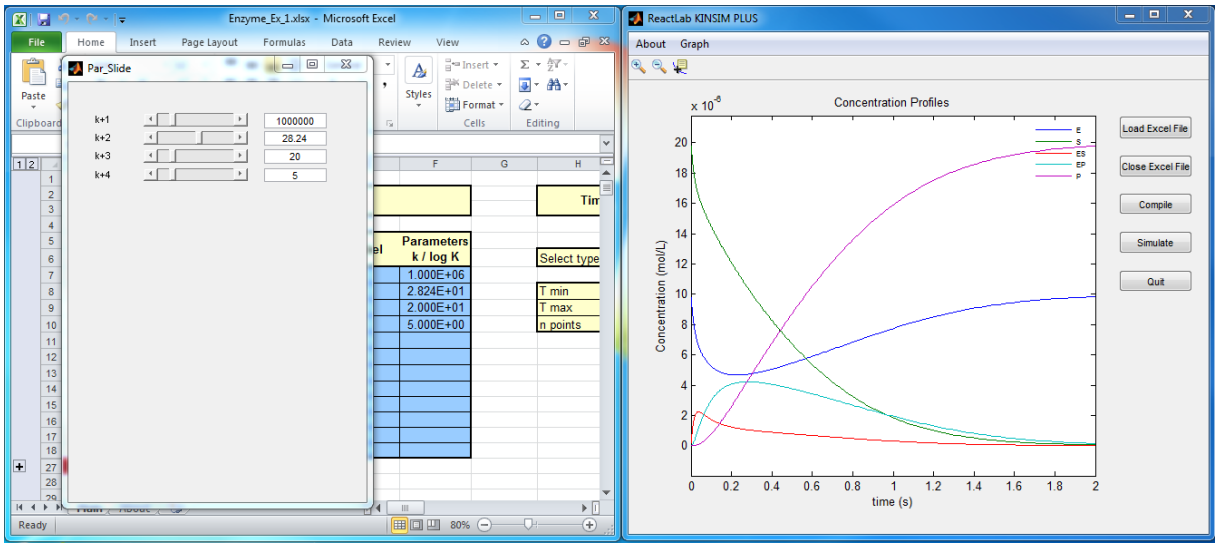


Figure 23 The KINSIM GUI, Excel workbook example in the background and the Parameter Slider Dialog box.

The main use of the parameter sliders is to conveniently observe how sensitive the concentration profiles are to the various parameters involved and to see the effects of adjustments to them interactively.

PLUS FINISH

NUMERICAL AND OTHER OPTIONS

A range of numerical calculation and other options are provided in the **'Main'** sheet that can be adjusted. There are also a number of software flags.

Numerical calculation options			
Numerical Integration		Equil. Speciation	
Stiff Solver	☑	Conv tol	1.00E-15
		Max iter	9.90E+01
Abs tol	1.00E-10		
Rel tol	1.00E-07		
		Miscellaneous	
		logKw	-14.00

Figure 24: The array of option definitions

Numerical

Several of the numerical options and are included for completeness rather than intended for routine customer use.

Numerical Integration

- Stiff solver:
  This is switches the numerical integration routine used during the calculation of concentration profiles during update or fitting. It is relevant when very large differences in rates of change occur in concentration profiles. Particularly with multistep reactions with fast second order interactions. The standard solver is a 4^{th} order Runga-Kutta which is usually reliable and fast, but under stiff conditions can slow down significantly. If the numerical integration takes too long ReactLab issues a warning and suggest a switch the stiff solver. If you suspect you are facing a stiff problem select the Stiff solver option or indeed select it by default prior to saving the workbook.
- Abs tol/Rel tol:
  Used to determine tolerances in the numerical integration calculations.

Equilibrium Speciation

- Conv tol/Max Iter:
  Used for speciation calculations by Newton Raphson algorithm. Only relevant for models with rapid equilibria.

Miscellaneous

- logKw:
  This is an advanced feature and allows adjustment of the log of the ionic product of water Kw ($1.00x10^{-14}M^2$) if required. LogKw is -14 for pure water at room temperature but changes with mixed solvents, ionic strength and temperature. This equilibrium is included when modeling reactions involving protonation equilibria to correctly deal with [H] and therefore pH. See below for more details.

HANDLING PROTONATION EQUILIBRIA, [H], [OH] AND KW

In some cases reaction models in aqueous solution may include protonation equilibria. In such instances the autoprotolysis of water has to be taken into account. This is done automatically by ReactLab™ if any of the components in the system is identified as 'H' which is interpreted as a proton. This is one of the very few rules about the names of components or species in ReactLab™. As a consequence of the presence of protons it is assumed that the reaction is performed in aqueous solution and the equilibrium

$$H^+ + OH^- \underset{}{\overset{K_W}{\rightleftharpoons}} H_2O$$

is internally added to the present model. Further the hydroxide, OH, will be added automatically to the list of species.

The ionic product K_w is represented as:

$$K_W = [H^+]\,[OH^-]$$

The unionized water is omitted from the expression as its concentration is essentially constant. The value of K_W is then $1.00x10^{-14}$ M^2 for pure water at room temperature. If measurements are taken at a different temperature, solvent or ionic strength the correct value for $logK_w$ is stored in cell 'I55' of the '**Main**' worksheet.

If protons are involved in a process and the autoprotolysis of water should not be implemented automatically, an easy option is to call it 'P' or anything but 'H'.

MODEL ENTRY SYNTAX RULES

- A kinetic reaction step is indicated by the symbol ">", e.g.:

  A+B>C

- The parameter associated with ">" is a rate constant (1^{st} or 2^{nd} order).
- Reversible kinetic steps (slow equilibria) are expressed over two lines i.e.

  A+B>C

  C>A+B

  In this case both forward and reverse rate constants are independently defined and can be fitted.

- A rapid (instantaneous) equilibrium is indicated by "=".
- The parameter associated with "=" is an equilibrium constant K. This can be fitted but the microscopic forward and reverse rates are not accessible.
- The parameter when "=" is used for an equilibrium is log10 of the association equilibrium constant, logK. Note this means it can be negative or positive depending on whether the equilibrium constant itself is less or greater than 1.
- Each equilibrium can only have one product.
- No two equilibria can have the same product.
- Reactants and products within a particular step are combined using the "+" symbol.
- Species names can be single or multiple characters.
- The stoichiometries of species in a particular step are indicated by an integer to the left of the species name (no integer is assumed to mean a stoichiometry of 1).
- The species name 'H' is treated as a special case and if present will enable the automatic incorporation of calculations to account for the autoprotolysis of water. In this situation 'OH' will also be added to the participating species list.
- In this version of ReactLab™ OH cannot participate in a kinetic step – only in the auto- equilibrium with H (above)

Thus equilibria can be incorporated into a reaction model in two ways. They can be expressed as kinetically observable forward and back reactions, each by using ">" symbol, allowing the individual rates to be potentially fitted. Alternatively they can be expressed as equilibria, using the "=" symbol, where the equilibration is instantaneous at any particular time. In this case the equilibrium is defined by its equilibrium constant and the concentrations of the equilibrium species at a particular time are determined using the law of mass action. This mode of calculation is particularly suited to equilibria where equilibration is so rapid that it cannot be observed (e.g. protonation equilibria).

EXAMPLES

EXAMPLE 1: SIMPLE SECOND ORDER REACTION A+B→C (AplusBtoC.xlsx)

In this first example we will go through the steps of setting up the model, the compilation, the definition of the constants, the initial concentrations and the time vector of reaction times at which the concentrations will be computed. The first example is a simple second order reaction:

$$\mathbf{A + B} \xrightarrow{k} \mathbf{C}$$

Loading the Excel template

The file 'ReactLab_KINSIM_Template.xlsx' is the template into which the information required for the simulation is entered. After these entries, it is saved under a different name and then ready for re-opening for further changes and simulations.

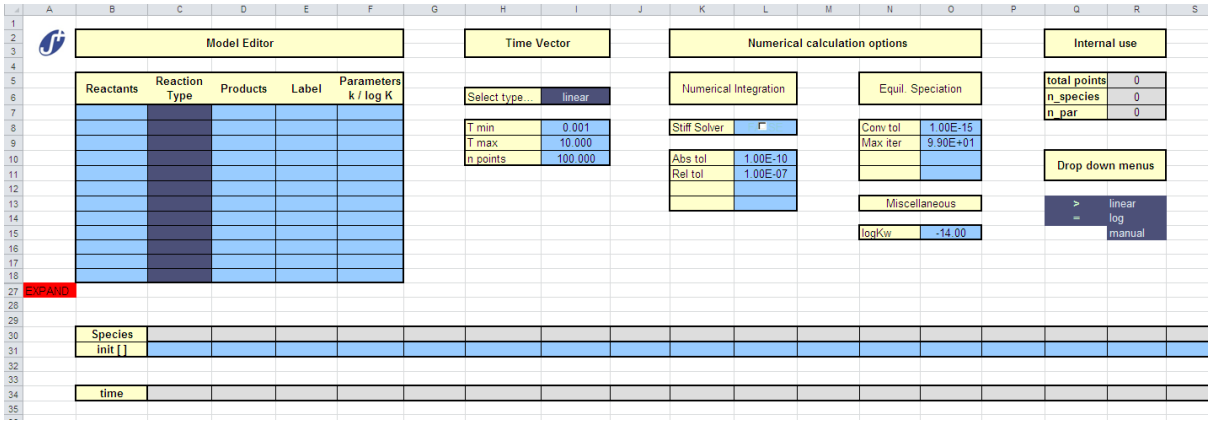


Figure 25: The Excel Template, ready to receive the relevant information

The Model

The first step is to define the reaction mechanism or the model; this is done by entering the information into the appropriate cells of the Excel spreadsheet. The ReactLab model entry is very flexible and allows the notation that chemists would use on paper.

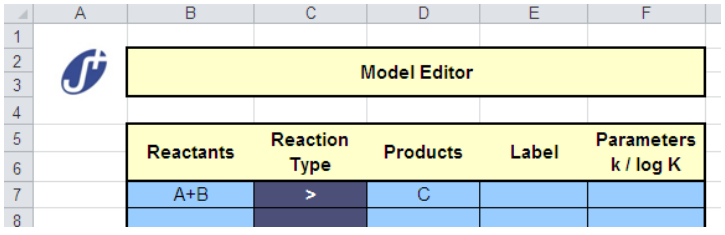


Figure 26: Model entry for the reaction A+B→C

Compilation

Compilation translates the model as defined in the spreadsheet into the internal code required by the algorithms

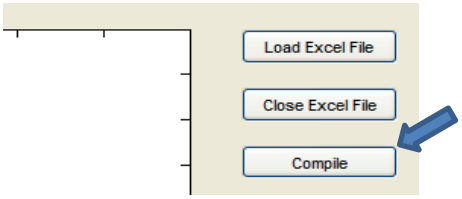


Figure 27: The compile button

Compilation also defines the list of all species in row 30 of the spreadsheet, see Figure 28.

Entry of the rate and equilibrium constants and the initial concentrations

The rate constants are defined for each reaction in column F and the initial concentrations for all species are entered in row 31 under the species names. Note that the initial concentrations are defined for time zero, not the first time in the time vector.

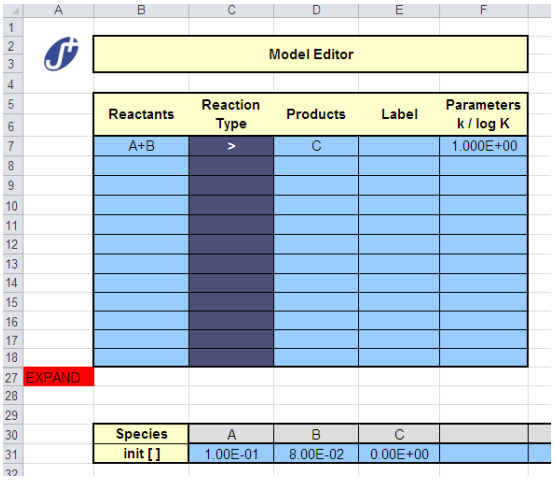


Figure 28: Entry of the rate constant in line with the reaction and of the initial concentrations under the species names

The time vector

There are three options to define the time vector, the Select type drop-down window allows the choice between linear, logarithmic, and manual definition.

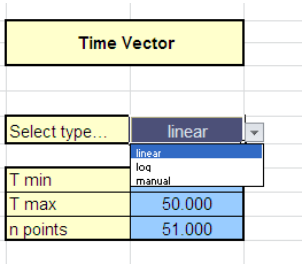


Figure 29: Three options to define the time vector

- **Linear**

In the linear mode, *n_points* are equidistantly arranged between the minimum and maximum times, *T_min* and *T_max*.

- **Logarithmic**

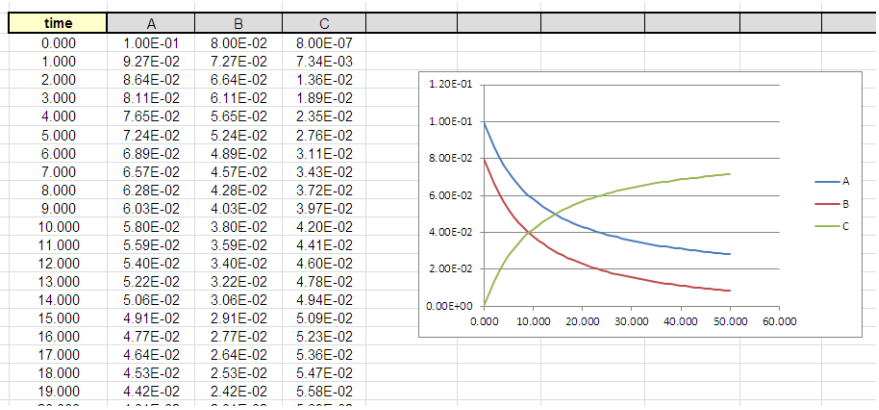
In the logarithmic mode, *n_points* are arranged between *T_min* and *T_max* in such a way that each time point is a constant factor times the preceding time. Note that *T_min* cannot be zero.

- **Manual**

Any arbitrary time vector with increasing points can be defined in the manual mode.

Simulate

Clicking the Simulate button creates the concentration profiles for all species which are written into the spreadsheet. Any additional calculation, manipulation or plotting can be done in the normal Excel way.



time	A	B	C
0.000	1.00E-01	8.00E-02	8.00E-07
1.000	9.27E-02	7.27E-02	7.34E-03
2.000	8.64E-02	6.64E-02	1.36E-02
3.000	8.11E-02	6.11E-02	1.89E-02
4.000	7.65E-02	5.65E-02	2.35E-02
5.000	7.24E-02	5.24E-02	2.76E-02
6.000	6.89E-02	4.89E-02	3.11E-02
7.000	6.57E-02	4.57E-02	3.43E-02
8.000	6.28E-02	4.28E-02	3.72E-02
9.000	6.03E-02	4.03E-02	3.97E-02
10.000	5.80E-02	3.80E-02	4.20E-02
11.000	5.59E-02	3.59E-02	4.41E-02
12.000	5.40E-02	3.40E-02	4.60E-02
13.000	5.22E-02	3.22E-02	4.78E-02
14.000	5.06E-02	3.06E-02	4.94E-02
15.000	4.91E-02	2.91E-02	5.09E-02
16.000	4.77E-02	2.77E-02	5.23E-02
17.000	4.64E-02	2.64E-02	5.36E-02
18.000	4.53E-02	2.53E-02	5.47E-02
19.000	4.42E-02	2.42E-02	5.58E-02

Figure 30: The numerical output of the Simulation with an example graph

The concentration [A] and [B] decrease from their initial values while [C] increases accordingly. After 50 sec the reaction is still proceeding, it is easy to re-define the time vector and simulate a longer time span. After 200 sec, the concentration of the minor starting material has essentially reached zero and the reaction is finished.

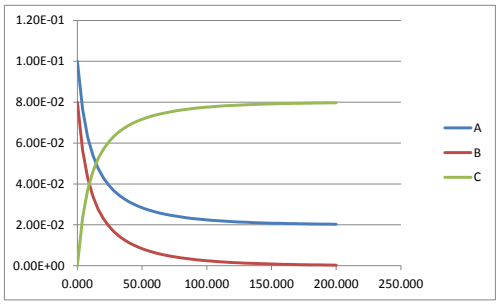


Figure 31: The same reaction simulated for 200 sec.

EXAMPLE 2: REVERSIBLE SECOND ORDER REACTION A+B↔C (AplusBeqC.xlsx)

The second order reaction of Example 1 does not require ReactLab, the concentration profiles can be calculated analytically.

$$[A] = \frac{[A]_0([B]_0 - [A]_0)}{[B]_0 e^{([B]_0 - [A]_0)kt} - [A]_0} \qquad ([A]_0 > [B]_0)$$

We invite the reader to implement the equations in Excel and compare with the output of ReactLab.

If the reaction is reversible numerical integration, as performed by ReactLab, is mandatory. The model is defined in two independent reactions.

$$A + B \underset{k_-}{\overset{k_+}{\rightleftharpoons}} C$$

Reactants	Reaction Type	Products	Label	Parameters k / log K
A+B	>	C	k+	1.000E+00
C	>	A+B	k-	1.000E-01

Figure 32: The ReactLab model definition for a reversible second order reaction

The concentration profiles are different and the equilibrium position is reached in some 20 sec. The equilibrium constant for the rate constants above is of course 10, $K=k_+/k_-$.

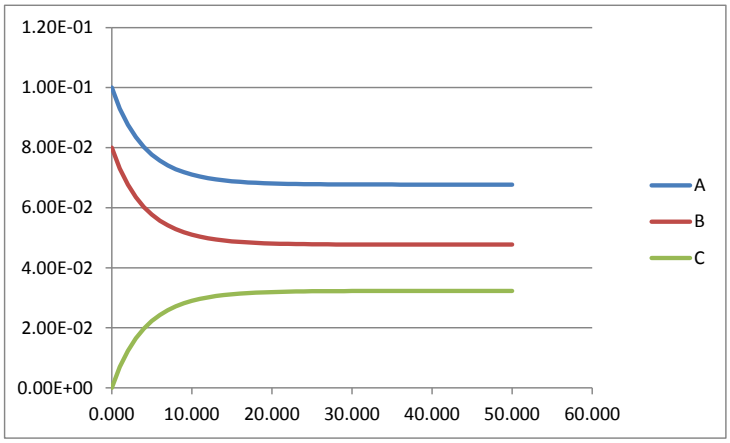


Figure 33: The concentration profiles for the reversible second order reaction

ENZYME KINETICS

EXAMPLE 3: ENZYME KINETICS (Enzyme_1.xlsx)

Figure 34 displays the 'Main' worksheet (*Enzyme_1.xlsx*) which models the interaction of the substrate **S** with the enzyme **E** and subsequent irreversible product formation.

$$\mathbf{E}+\mathbf{S} \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} \mathbf{ES} \xrightarrow{k_2} \mathbf{EP} \xrightarrow{k_3} \mathbf{E}+\mathbf{P}$$

To see the simulation in the KINSIM GUI select 'Compile' and then 'Simulate' in the GUI. This is necessary to initialise ReactLab with the model in the workbook and then simulate it according to the initial parameters and timebase.

In the first reversible step the enzyme reacts with the substrate to form the intermediate **ES** which subsequently forms, in an irreversible step, the enzyme product complex **EP** which in turn decays, also irreversibly, to release the product **P** and the enzyme which can start another cycle.

The advantage of KINSIM is that it can be used to simulate enzyme kinetics under both steady state conditions (see Example 4 below) and to investigate the pre-steady state behaviour when substrate and enzyme first interact. Under steady state conditions high substrate and catalytic enzyme concentrations mean the enzyme bound intermediates are rapidly saturated and remain approximately constant until the substrate is exhausted. Historically this was the only practical way enzyme reactions could be studied and provided the foundation of the development of the steady state assumption and Michealis-Menten kinetic analysis quantifying enzyme behaviour in terms of K_M and k_{cat}. No information was available to elucidate the details and specific kinetic events of the underlying enzyme mechanism. We will illustrate this further in later examples.

One of the reasons analysis was restricted in this way was because of the limited availability of purified enzyme and insufficiently sensitive kinetic detection technologies. When protein purification improved and enzymes could be produced in larger amounts experiments could be conducted which allowed the formation of enzyme intermediates during the pre-steady state phase to be observed. This also required the development of instrumental techniques such a stopped-flow coupled in particular to highly sensitive fluorescence detection. For further information on the emergence of pre-steady state kinetics and a thorough discussion of the evolution of the study of enzyme kinetics the reader is referred to the references of Fersht and Gutfreund.

Fortunately, simulation and modelling of these processes is not hampered by experimental constraints. In a so-called single-turnover experiment we can 'observe' the kinetics during an experiment where the substrate and enzyme concentrations are similar ($[E]_0=10^{-5}$M, $[S]_0=2\times10^{-5}$M). For the given initial concentrations ($[E]_0=10^{-5}$M, $[S]_0=2\times10^{-5}$M) and rate constants ($k_1=10^6 M^{-1}s^{-1}$, $k_{-1}=10s^{-1}$, $k_2=2s^{-1}$ and $k_3=4s^{-1}$) the first fast step is the formation of the **ES** complex, this phase of the reaction takes about 100 msec. The next phase consists of the transformation of the **ES** into the **EP** intermediate which in turn reacts further to release the product. The complete transformation of the substrate takes about 5 sec; at that time

the enzyme is also released completely and reaches the initial concentration while the product reaches the initial concentration of the substrate.

ReactLab KINSIM calculates the concentration profiles for the predefined timespan and stores them in the columns B to G; they can be plotted in Excel in the usual way.

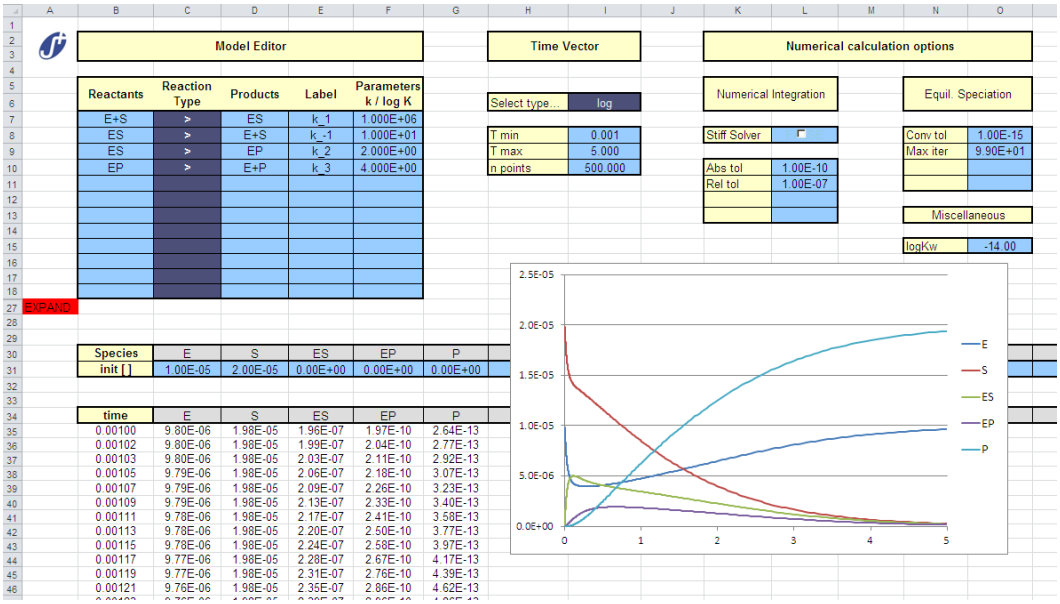


Figure 34: The Enzyme_1.xlsx example workbook

It is worthwhile noting that the concentrations seen in the above example are often not observable in traditional absorption spectroscopy. In contrast, fluorescence emission spectroscopy can be much more sensitive and for some substrates the above reactions can be observed (see Gutfreund).

EXAMPLE 4: ENZYME KINETICS REVISITED (Enzyme_2.xlsx)

In this example we continue with the same enzymatic mechanism but explore its bahaviour under different conditions.

$$\mathbf{E} + \mathbf{S} \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} \mathbf{ES} \xrightarrow{k_2} \mathbf{EP} \xrightarrow{k_3} \mathbf{E} + \mathbf{P}$$

The initial concentration of enzyme, **E**, and substrate, **S**, were previously chosen to demonstrate the initial interactions to form the first and second intermediates, **ES** and **EP**, and the final product, **P**. This was a so-called single-turnover or pre-steady-state experiment. Such small concentrations for the substrate enable the pre-steady state transient kinetics to be observed and the mechanism dissected but now we will see how the system behaves under more biologically relevant steady state conditions.

In this example we use the same rate constants but have an initial substrate concentration of 0.01 M, the initial enzyme concentration is still 10^{-5} M. Plotting the concentration of substrate and product reveals the classical pseudo-zeroth order kinetics: a linear decrease

of substrate with the concomitant linear increase in the product concentration. This is the result of the limited amount of enzyme which turns over the substrate at a constant rate until it runs out. Under these conditions there is negligible free enzyme during this phase as it is all bound in the form of **ES** and **EP**, and the enzyme reaction is proceeding at the maximum rate possible. This is the V_{max} in Michaelis-Menten steady state terminology. The rate of continuous product formation during this steady state phase is known as the k_{cat} or turnover number of the enzyme ($=V_{max}/[\mathbf{E}]_0$) which quantifies how much substrate is converted to product per second.

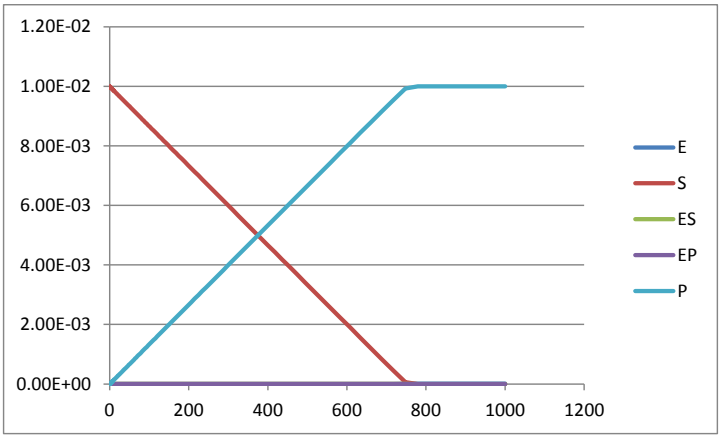


Figure 35: Pseudo first-order enzyme kinetics, linear decreased and increase of substrate and product

The next Figure reveals some interesting details of the simulation. Part a) only shows the profiles for the enzyme and the two intermediates (this requires a highly zoomed-in y-axis to be seen in the ReactLab GUI). Clearly visible is the long section where the concentrations of the intermediates are almost perfectly constant; this of course is the well-known steady-state situation. Part b) features a logarithmic time scale. The formation of the first intermediate takes about 1 msec after which the enzyme concentration reaches very low concentration it is almost quantitatively transformed into the first intermediate. After about 1 sec the transformation into the second intermediate has progressed to the steady-state concentrations.

This Figure also demonstrates the ease by which such plots can be created in Excel. We refer to user to many textbook and help files for Excel.

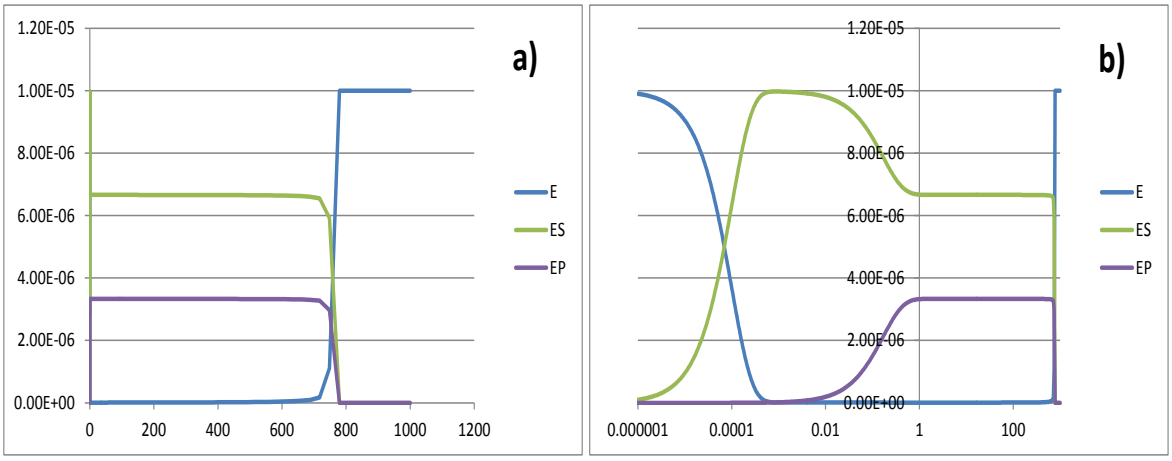


Figure 36: a) the concentration profiles of the intermediate enzyme complexes demonstrate the steady-state behavior; b) a logarithmic time scale reveals the very fast formation of the first intermediate and transformation into the second intermediate.

EXAMPLE 5: ENZYME KINETICS REVISITED AGAIN (Enzyme_3.xlsx)

The initial interaction between the enzyme and the substrate is very fast and normally not observable. It is then not possible to determine the individual values for the forward and back rate constants (k_1 and k_{-1}) and ***it makes sense to define this first step as an instantaneous equilibrium***. ReactLab allows the definition of such equilibria very straightforwardly. The '=' sign in the Reaction Type cell defines the reaction as an, instantaneous, equilibrium. Note that equilibrium constants in the parameter cells are always defined as their logarithms to the base of 10. $K_1 = k_1/k_{-1} = 10^6/10 = 10^5$; $\log K_1 = 5$.

$$\mathbf{E + S} \underset{}{\overset{\mathbf{K_1}}{\rightleftharpoons}} \mathbf{ES} \xrightarrow{\mathbf{k_2}} \mathbf{EP} \xrightarrow{\mathbf{k_3}} \mathbf{E + P}$$

Reactants	Reaction Type	Products	Label	Parameters k / log K
E+S	=	ES	K_1	5.000E+00
ES	>	EP	k_2	2.000E+00
EP	>	E+P	k_3	4.000E+00

Figure 37: Definition of the interaction between enzyme and substrate as an equilibrium

Comparing Figure 36 with Figure 38 reveals that the concentration profiles are essentially indistinguishable, apart from the first msec where the E+S interaction takes place for the kinetic definition. Here the establishment of the equilibration is instantaneous.

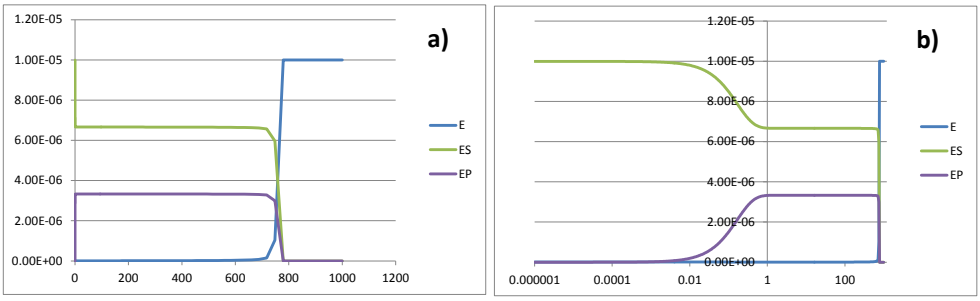


Figure 38: as Figure 36 with the first step defined as equilibrium

EXAMPLE 6: LOTKA-VOLTERRA PREDATOR-PREY MODEL (LOTKA_VOLTERRA_PREDATOR_PREY_MODEL.XLSX)

An interesting 'kinetic' model is the predator-prey 'reaction' developed by Lotka and Volterra. Imagine an island with a certain population of sheep and wolves. There are several 'reactions': in a first 'reaction' the sheep breed, relying on grass as their feed. The reaction is, of course, biologically not quite correct:

$$\mathbf{sheep} \xrightarrow{k_1} \mathbf{2\,sheep}$$

The wolves of the island also multiply but they need sheep as source of energy

$$\mathbf{wolf + sheep} \xrightarrow{k_2} \mathbf{2\,wolf}$$

(Note that ReactLab does not 'speak' English and would not understand the reaction wolf+sheep→2wolves.)

The wolf population is kept under control by wolves dying

$$\mathbf{wolf} \xrightarrow{k_3} \mathbf{dead}$$

The ReactLab entry is just as above

Reactants	Reaction Type	Products	Label	Parameters k / log K
sheep	>	2sheep	k1	2.000E+00
wolf + sheep	>	2wolf	k2	5.000E+00
wolf	>	dead	k3	5.000E+00

Figure 39: Model entry for the predator-prey model

In Excel it is straightforward to delete the 'concentration profile' of the dead wolves to reveal the perfect cyclic behavior of this 'reaction'.

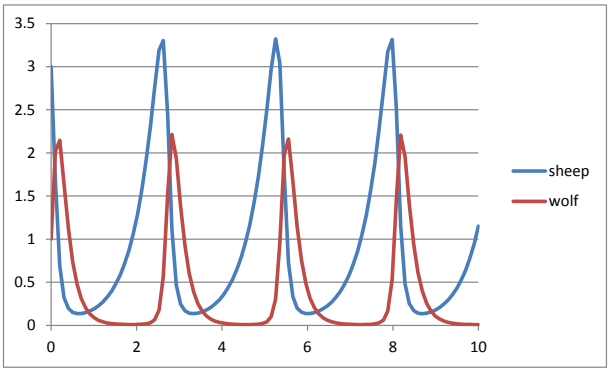


Figure 40: The completely cyclic behavior of the Lotka-Volterra predator-prey model

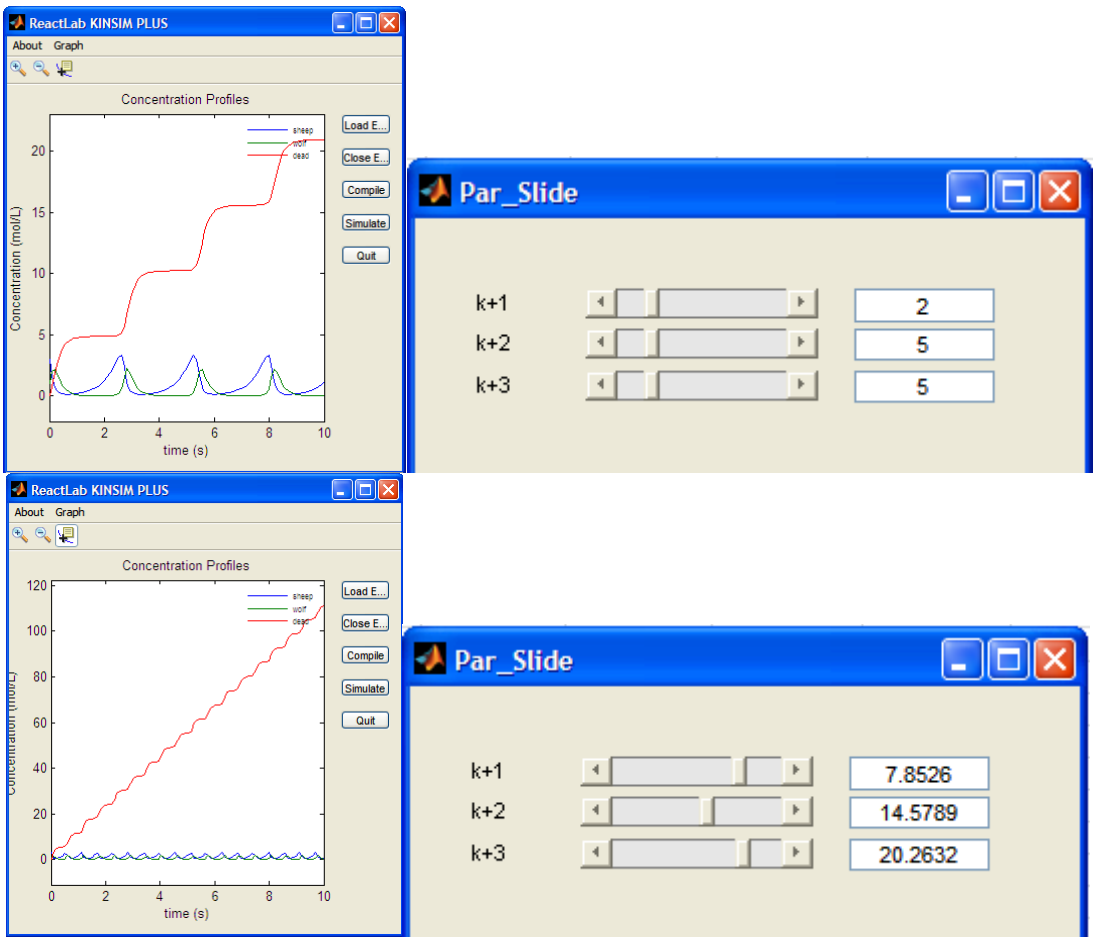


Figure 41: The ReactLab KINSIM window with the concentration profiles. The Parameter slide GUI of ReactLab KINSIM PLUS allows the convenient change of any of the rate constants

EXAMPLE 7: THE BELOUSOV-ZHABOTINSKY REACTION (Belousov-Zhabotinsky.xlsx)

The Belousov-Zhabotinsky reaction is a famous cyclic reaction. In contrast to the predator-prey model before this reaction one is real. However, the reaction mechanism is not completely understood and a simplified version that is usually used to describe the chemical reaction is chemically/stoichiometrically not quite correct.

The Belousov-Zhabotinsky (BZ) reaction involves the oxidation of an organic species such as malonic acid (MA) by an acidified aqueous bromate solution in the presence of a metal ion catalyst such as the $Ce^{(III)}/Ce^{(IV)}$ couple. At excess [MA] the stoichiometry of the net reaction is

$$2BrO_3^- + 3CH_2(COOH)_2 + 2H^+ \xrightarrow{\text{catalyst}} 2BrCH(COOH)_2 + 3CO_2 + 4H_2O$$

A reasonable approximation for the cyclic behavior results from the following reaction mechanism.

$$BrO_3^- + Br^- \xrightarrow{k_1} HBrO_2 + HOBr$$
$$BrO_3^- + HBrO_2 \xrightarrow{k_2} 2HBrO_2 + 2M_{ox}$$
$$HBrO_2 + Br^- \xrightarrow{k_3} 2HOBr$$
$$2HBrO_2 \xrightarrow{k_4} BrO_3^- + HOBr$$
$$MA + M_{ox} \xrightarrow{k_5} \tfrac{1}{2} Br^-$$

With rate constants and initial concentrations as defined in the spreadsheet in Figure 42 the cyclic behavior of Figure 43 results.

Reactants	Reaction Type	Products	Label	Parameters k / log K
BrO3+Br	>	HBrO2+HOBr	k_1	1.280E+00
BrO3+HBrO2	>	2HBrO2+2Mox	k_2	3.360E+01
HBrO2+Br	>	2HOBr	k_3	2.400E+06
2HBrO2	>	BrO3+HOBr	k_4	3.000E+03
MA+Mox	>	Bri	k_5	1.000E+00
2Bri	>	Br	k_6	1.000E+06

Select type...	linear
T min	0.000
T max	1000.000
n points	500.000

Species	BrO3	Br	HBrO2	HOBr	Mox	MA	Bri
init [ ]	6.30E-02	1.00E-02	0.00E+00	0.00E+00	2.00E-03	2.75E-01	0.00E+00

Figure 42: the Belousov-Zhabotinsky reaction in ReactLab

The numerical integration delivers the concentration profiles for all species. Figure 43 a) demonstrates the slow conversion of the malonic acid and bromate to HOBr. The oscillations for the remaining species are not visible as the concentrations are too low. Using a logarithmic concentration axis reveals the oscillations, Figure 43 b).

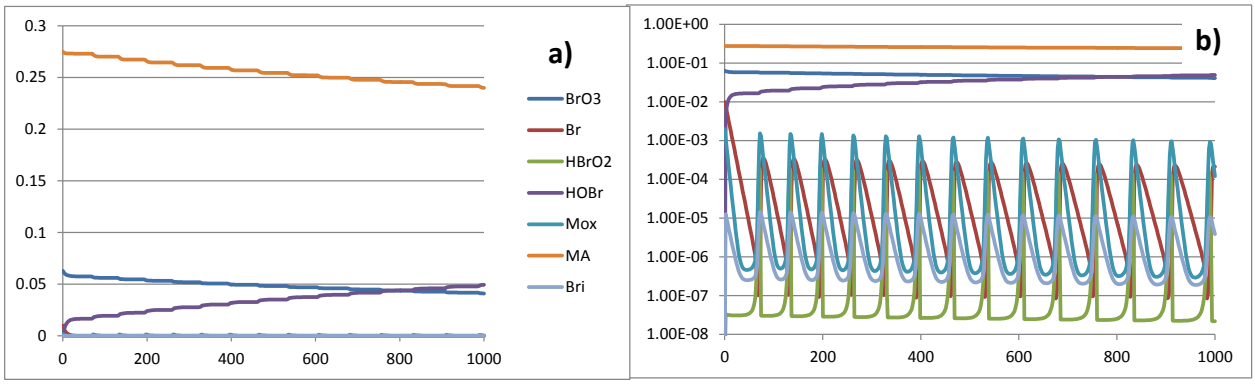


Figure 43: The concentration profiles for the Belousov-Zhabotinsky reaction with a linear and logarithmic y-axis

Note that this example and also the following one have been integrated numerically using the stiff solver, see NUMERICAL AND OTHER OPTIONS and Figure 24 for more information.

EXAMPLE 8: REACTION OF CU^{2+} WITH CYCLAM (Cu_cyclam.xlsx)

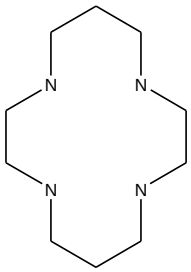
A unique capacity and strength of ReactLab is its ability to include instantaneous equilibria into its reaction model. The most important examples for such equilibria are of course protonation equilibria.

The reaction between a metal ion and an amine ligand in aqueous solution is strongly pH dependent as the ligand is involved in one or several protonation equilibria. Each differently protonated form will react with the metal ion with different rate constants.

The following example is taken from the literature, the publication where the above idea is published the first time

> *Analysis of reactions in aqueous solution at non-constant pH: No more buffers? Marcel Maeder, Yorck-Michael Neuhold, Graeme Puxty, Peter King Phys. Chem. Chem. Phys., 2003, 5, 2836-2841*

The reaction is between Cu^{2+} and cyclam which is a cyclic tetra-amine.



Cyclam exists in 5 differently protonated forms, L, LH, LH_2, LH_3, and LH_4. Each form reacts with Cu^{2+} with a different rate constant

$$Cu^{2+} + LH_x^{x+} \xrightarrow{k_{LHx}} CuL^{2+} + xH^+$$

The differently protonated forms are defined by their protonation constant

$$LH_{x-1}^{(x-1)+} + H^+ \xrightarrow{K_x} LH_x^x$$

In ReactLab the model definition and its rate and equilibrium constants as well as the initial concentrations are as follows.

Reactants	Reaction Type	Products	Label	Parameters k / log K
Cu+LH	>	CuL+H	k_LH	1.050E+06
Cu+LH2	>	CuL+2H	k_LH2	1.350E-01
L+H	=	LH	K_1	1.159E+01
LH+H	=	LH2	K_2	1.062E+01
LH2+H	=	LH3	K_3	1.560E+00
LH3+H	=	LH4	K_4	2.340E+00

Select type...	linear
T min	0.000
T max	30000.000
n points	100.000

Numerical Inte	
Stiff Solver	
Abs tol	1.
Rel tol	1.

Species	Cu	CuL	H	L	LH	LH2	LH3	LH4	OH
init [ ]	3.94E-03	0.00E+00	9.60E-03	4.33E-03	0.00E+00	0.00E+00	0.00E+00	0.00E+00	0.00E+00

Figure 44: The model for the reaction of Cu^{2+} with cyclam.

The modelling results in the same concentration profiles as in Figure 3(b) of the above publication.

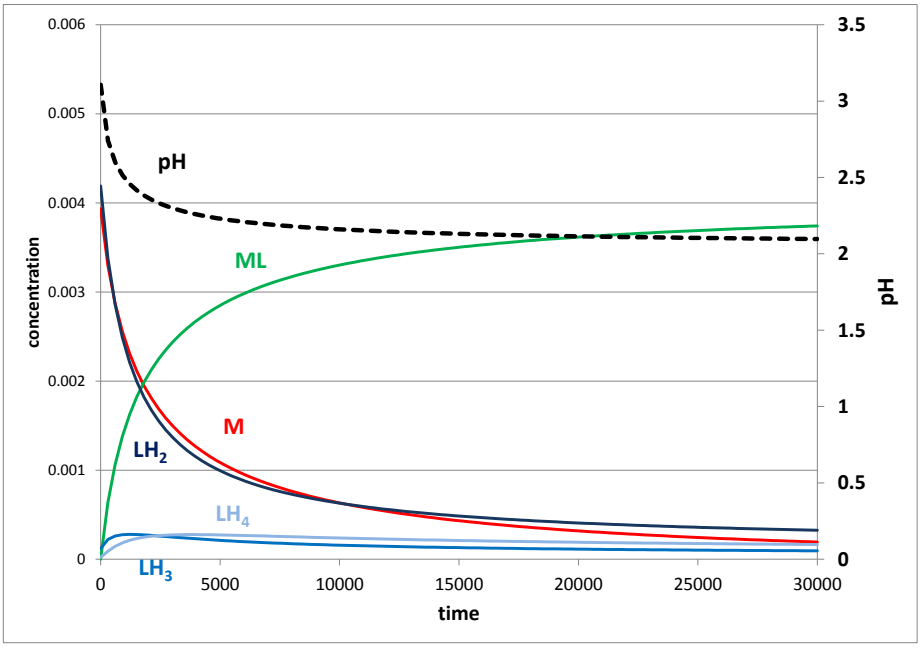


Figure 45: The resulting concentration profiles

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